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Effect of ω-conotoxin MVIIA and Phα1β on paclitaxel-induced acute and chronic pain

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ABSTRACT

The treatment with the chemotherapeutic agent paclitaxel produces a painful peripheral neuropathy, and is associated with an acute pain syndrome in a clinically significant number of patients. However, no standard therapy has been established to manage the acute pain or the chronic neuropathic pain related to paclitaxel. In the present study, we evaluated the analgesic potential of two N-type voltage-gated calcium channel (VGCC) blockers, ω-conotoxin MVIIA and Phα1β, on acute and chronic pain induced by paclitaxel. Adult male rats were treated with four intraperitoneal injections of paclitaxel (1 + 1 + 1 + 1 mg/kg, in alternate days) and the development of mechanical hyperalgesia was evaluated 24 h (acute painful stage) or 15 days (chronic painful stage) after the first paclitaxel injection. Not all animals showed mechanical hyperalgesia 24 h after the first paclitaxel injection, but those that showed developed a more intense mechanical hyperalgesia at the chronic painful stage. Intrathecal administration (i.t.) of ω-conotoxin MVIIA (3–300 pmol/site) or Phα1β (10–300 pmol/site) reduced the mechanical hyperalgesia either at the acute or at the chronic painful stage induced by paclitaxel. When administered at the acute painful stage, ω-conotoxin MVIIA (300 pmol/site, i.t.) and Phα1β (300 pmol/site, i.t.) prevented the worsening of chronic mechanical hyperalgesia. Furthermore, Phα1β (30-300 pmol/site, i.t.) elicited less adverse effects than ω-conotoxin MVIIA (10-300 pmol/site, i.t.). Taken together, our data evidence the involvement of N-type VGCC in pain sensitization induced by paclitaxel and point out the potential of Phα1β as a safer alternative than ω-conotoxin MVIIA to treat the pain related to paclitaxel.

1. Introduction

Paclitaxel is a chemotherapeutic agent commonly used in solid tumors (Rowinsky et al., 1993; Polomano et al., 2001). The main side effect associated with long-term use of paclitaxel is a chronic peripheral neuropathy, characterized by mechanical hyperalgesia, tingling and numbness affecting the distal extremities (Rowinsky et al., 1993; Forsyth et al., 1997; Dougherty et al., 2004; Woolf et al., 2008). The onset of sensory symptoms during paclitaxel treatment usually leads to a reduction in the dose or interruption of therapy, which can negatively affect cancer therapy outcomes (Polomano and Bennett, 2001; Dougherty et al., 2004). Moreover, it has been recently reported that paclitaxel is also associated with an acute pain syndrome that develops in the first days of treatment, and affects a large proportion of patients (Loprinzi et al., 2007, 2011; Reeves et al., 2012). Furthermore, the acute pain experienced by some patients in the beginning of the treatment with paclitaxel appears to be somehow related to the severity of the later neuropathic pain (Loprinzi et al., 2011; Reeves et al., 2012). The chronic neuropathic pain induced by paclitaxel has been studied using animal models (Polomano et al., 2001; Naguib et al., 2012), however, to our knowledge, no preclinical studies have addressed the acute pain induced by paclitaxel or its influence in chronic neuropathic pain.

Currently, there are no standard therapies to prevent or minimize the pain related to paclitaxel (Rowinsky et al., 1993; Gordon et al., 1997; Wasserheit et al., 1996; Loprinzi et al., 2011; Reeves et al., 2012). Nevertheless, several lines of evidence suggest the involvement of voltage-gated calcium channels (VGCC) in pain arising from nerve damage (Piekarz et al., 2012; Arcos et al., 2013; Kukkar et al., 2013), including those induced by chemotherapeutic agents (Flatters and

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Bennett, 2004; Siau et al., 2006; Xiao et al., 2007; Saif and Hashmi, 2008; Kawakami et al., 2012). Furthermore, a N-type VGCC blocker peptide derived from the venom of the cone snail *Conus magus*, the ω-conotoxin MVIIA, had its synthetic form, ziconotide, recently approved for the treatment of severe chronic pain, including neuropathic pain refractory to other therapies (Staats et al., 2004; Prommer, 2006). However, whether ziconotide alleviates the pain related to paclitaxel treatment is yet to be investigated.

The clinical use of ziconotide, despite having confirmed the good analgesic efficacy of ω-conotoxin MVIIA, has revealed to cause serious adverse effects (Penn and Paice, 2000; Rauck et al., 2009; Achim et al., 2010; Maier et al., 2011). We have been studied the analgesic potential of a peptide toxin isolated from the venom of the Brazilian spider *Phoneutria nigriventer*, named Phα1β, which inhibits VGCC, mainly the N-type calcium currents (Vieira et al., 2005) in several animal models of pain (Souza et al., 2008; de Souza et al., 2011, 2013; Castro-Junior et al., 2013; Rigo et al., 2013). The Phα1β has shown antinociceptive effect with a larger therapeutic window when comparing to ω-conotoxin MVIIA (Souza et al., 2008; Castro-Junior et al., 2013; Rigo et al., 2013).

The primary goal of the present study was evaluate the analgesic potential of ω-conotoxin MVIIA and Phα1β in the acute and chronic neuropathic pain induced by paclitaxel. We showed that both VGCCs reduced the acute and chronic mechanical hyperalgesia induced by paclitaxel, but Phα1β produced less adverse effects than ω-conotoxin MVIIA. In addition, we described a positive relation between the development of acute pain and the degree of chronic neuropathic pain induced by paclitaxel that can be controlled by spinal injection of both VGCC blockers.

2. Materials and methods

2.1. Animals

Male adult Wistar rats weighing 250–300 g were used. All experimental procedures were approved by the ethics committee of the Federal University of Santa Maria (process number: 23081.005024/2010-88), and were carried out in accordance with the guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals (Zimmermann, 1983).

2.2. Drugs

Phα1β was purified from the venom of the Brazilian spider *P. nigriventer*, as previously described (Cordeiro et al., 1993). The N-type VGCC blocker ω-conotoxin MVIIA was purchased from Latoxan (Valence, France). Phα1β and ω-conotoxin MVIIA were dissolved in phosphate-buffer saline (PBS; pH 7.4). Paclitaxel (6 mg/mL paclitaxel in Cremophor EL and dehydrated ethanol) was purchased from Glenmark (Buenos Aires, Argentina), and was dissolved in saline solution (NaCl, 0.9%).

2.3. Paclitaxel-induced acute and chronic pain model

Different group of rats received one or four intraperitoneal (i.p.) injections of paclitaxel (1 mg/kg), injected on alternate days (Polomano et al., 2001). Acute pain was evaluated from 24 to 72 h after a single injection of paclitaxel. Chronic pain was evaluated at 7, 15, 22, and 30 days after the first of four paclitaxel injections. Acute pain and chronic pain were evaluated using the von Frey test, which measures mechanical sensitivity. Mechanical hyperalgesia was defined as a reduction of the baseline paw withdrawal threshold of ≥50% after paclitaxel treatment.

2.4. Behavioral tests

2.4.1. Von Frey test

Mechanical threshold was measured by applying von Frey filaments on the plantar surface (sciatic nerve territory) of the rat's hindpaw, using the up–down method, as described previously (Chaplan et al., 1994; Dixon, 1980). Briefly, rats were first acclimatized (1–2 h) in individual clear Plexiglas boxes on an elevated wire mesh platform to allow access to the plantar surface of the hind paws. Von Frey filaments of increasing stiffness (6–100 g) were applied to the hind paw plantar surface of the animals with a pressure high enough to bend the filament. The absence of a paw lifting after 5 s led to the use of the next filament with increasing weight, whereas paw lifting indicated a positive response and led to the use of the next weaker filament. This paradigm continued for a total of 6 measurements, including the one before the first paw-lifting response had been made, or until 4 consecutive positive or 4 consecutive negative responses occurred. The 50% mechanical paw withdraw threshold (PWT) response was then calculated from the resulting scores as described previously by Dixon. The PWT was expressed in grams (g).

2.4.2. Adverse effects assessment

Adverse effects elicited by the treatments, such as serpentine-like tail movement, body shaking and allodynia were assessed and quantified using a 7-point scale, as previously described (Smith et al., 2002; Malmberg and Yaksh, 1994).

2.5. Experimental design

The ω-conotoxin MVIIA and Phα1βwere administered by intrathecal (i.t.) route, as previously described (Dalmolin et al., 2011).

First, we evaluated the time-course of paclitaxel-induced acute and chronic pain in two separated group of rats. In one group of rats, the mechanical hyperalgesia was evaluated from 24 to 72 h after a single injection of paclitaxel (1 mg/kg, i.p.). In another group, rats received 4 injections of paclitaxel (1 + 1 + 1 + 1 mg/kg, i.p., in alternate days) and after 7, 15, 22, and 30 days of the first paclitaxel injection they had their mechanical threshold examined.

Next, the time-course and the dose–response curve of antinociceptive and adverse effects caused by i.t. treatment with ω-conotoxin MVIIA (3–300 pmol/site) and by Phα1β (10–300 pmol/site) were evaluated on acute pain (24 h after a single injection of paclitaxel) and chronic neuropathic pain (15 days after the first injection, in rats receiving 4 paclitaxel injections) induced by paclitaxel, in separated group of rats.

The next step was to investigate whether N-type VGCC blockade in the acute painful stage affects the mechanical hyperalgesia in the chronic painful stage. In brief, rats had their mechanical sensitivity evaluated before and 24 h after the first of four paclitaxel injections (1 mg/kg, i.p.). Those rats that showed mechanical hyperalgesia (reduction of the baseline PWT ≥ 50%), were considered as part of the group that developed acute pain, called acute pain affected group. The rats that did not show mechanical hyperalgesia were classified as acute pain non-affected group. Once the mechanical hyperalgesia was established, the acute pain affected group was treated with PBS (10 μl/site, i.t.), Phα1β (300 pmol/site, i.t.), or ω-conotoxin MVIIA (300 pmol/site, i.t.) and evaluated from 15 to 360 min post-treatment in the von Frey test. Then, both acute pain affected and non-affected groups continued receiving the next three paclitaxel injections in alternate days, and had their mechanical sensibility re-evaluated 15 days after the first injection, at the chronic painful stage. At this stage, those rats showing mechanical hyperalgesia were treated with PBS (10 μl/site, i.t.), Phα1β (300 pmol/site, i.t.), or ω-conotoxin MVIIA (30 pmol/site, i.t.), and had their mechanical threshold evaluated from 15 to 360 min. A schematic representation of the experimental design can be found in Fig. 1.

In all experiments, the rats were randomly assigned to individual experimental groups and the subsequent behavioral tests were performed by an experimenter blind to the treatment conditions. Each experiment was performed at least two times.